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60/190,288 17 March 2000 (17.03.2000) US

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(74) Agents: YEAGER, Sally, S. et al.; Alcon Research, Ltd., 6201 South Freeway, Fort Worth, TX 76134 (US).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/70207 A3

(54) Title: $5HT_2$ AGONISTS FOR CONTROLLING IOP AND TREATING GLAUCOMA

(57) Abstract: Compositions and methods for controlling intraocular pressure and treating glaucoma using 1-(2-aminopropyl)-indazol-6-ol are disclosed.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/31246

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/416 A61P27/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	OSBORNE N N: "SEROTONIN AND MELATONIN IN THE IRIS/CILIARY PROCESSES AND THEIR INVOLVEMENT IN INTRAOCULAR PRESSURE" ACTA NEUROBIOLOGIAE EXPERIMENTALIS, WARSZAW, PL, vol. 54, no. SUPPL, 1994, pages 57-64, XP000878634 ISSN: 0065-1400 abstract page 61, left-hand column, line 20 -page 62, left-hand column, line 2 ---	1-12
Y	WO 98 30548 A (TSUKAMOTO SHIN ICHI ;KUBOTA HIDEKI (JP); MAENO KYOICHI (JP); SHIMA) 16 July 1998 (1998-07-16) cited in the application abstract; example 46 ---	1-12
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 November 2001	03/12/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Villa Riva, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 01 40183 A (ALCON UNIVERSAL LTD ;DANTANARAYANA ANURA P (US); MAY JESSE A (US);) 7 June 2001 (2001-06-07) abstract; examples 6,7,16-18 -----	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int   tional Application No
PCT/US 00/31246

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9830548	A	16-07-1998	AU	5343298 A	03-08-1998
			WO	9830548 A1	16-07-1998
WO 0140183	A	07-06-2001	AU	2042301 A	12-06-2001
			WO	0140183 A1	07-06-2001

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(30) Priority Data:
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WO 01/70207 A2

(54) Title: $5HT_2$ AGONISTS FOR CONTROLLING IOP AND TREATING GLAUCOMA

(57) Abstract: Compositions and methods for controlling intraocular pressure and treating glaucoma using 1-(2-aminopropyl)-indazol-6-ol are disclosed.

$5HT_2$ AGONISTS FOR CONTROLLING IOP AND TREATING GLAUCOMA

The present invention is directed to the use of 1-(2-aminopropyl)-indazol-6-ol for lowering and controlling intraocular pressure (IOP) and treating glaucoma.

Background of the Invention

The disease state referred to as glaucoma is characterized by a permanent loss of visual function due to irreversible damage to the optic nerve. The several morphologically or functionally distinct types of glaucoma are typically characterized by elevated IOP, which is considered to be causally related to the pathological course of the disease. Ocular hypertension is a condition wherein intraocular pressure is elevated but no apparent loss of visual function has occurred; such patients are considered to be a high risk for the eventual development of the visual loss associated with glaucoma. Some patients with glaucomatous field loss have relatively low intraocular pressures. These so called normotension or low tension glaucoma patients can also benefit from agents that lower and control IOP. If glaucoma or ocular hypertension is detected early and treated promptly with medications that effectively reduce elevated intraocular pressure, loss of visual function or its progressive deterioration can generally be ameliorated. Drug therapies that have proven to be effective for the reduction of intraocular pressure include both agents that decrease aqueous humor production and agents that increase the outflow facility. Such therapies are in general administered by one of two possible routes, topically (direct application to the eye) or orally.

There are some individuals who do not respond well when treated with certain existing glaucoma therapies. There is, therefore, a need for other topical therapeutic agents that control IOP.

The compound, 1-(2-aminopropyl)-indazol-6-ol, is disclosed in WO98/30548. Example 46 within the application discloses the S-enantiomer of 1-(2-aminopropyl)-indazol-6-ol. The utility cited in the application is for treating central nervous system

diseases, such as, sexual disorders, genital insufficiency, appetite regulation disorders, anxiety, depression, and sleep disorders. No ophthalmic indications are disclosed.

Summary of the Invention

The present invention is directed to compositions of 1-(2-aminopropyl)-indazol-6-ol and its use for lowering and controlling IOP and treating glaucoma.

Description of Preferred Embodiments

Surprisingly, it has been found that 1-(2-aminopropyl)-indazol-6-ol ("Compound") and its S-(+)-isomer, when dosed at 300 μg, in the lasered monkey model of ocular hypertension causes a significant decrease in IOP as shown in the table set forth below. Intraocular pressure was determined with an Alcon Pneumatonometer after light corneal anesthesia with 0.1% proparacaine. Eyes were washed with saline after each measurement. After a baseline IOP measurement, test compound was instilled in one 30 μL aliquot to the right eyes only of nine cynomolgus monkeys. Vehicle was instilled in the right eyes of six additional animals. Subsequent IOP measurements were taken at 1, 3, and 6 hours. A compound is considered efficacious in this model of ocular hypertension if there is a decrease in the baseline IOP of the lasered eye (O.D.) of at least 20% following topical administration.

IOP Response to 1-(2-Aminopropyl)-indazol-6-ol and its S(+)-isomer

	Dose (μg)	Baseline IOP (mmHg)	IOP Response % Change (ΔmmHg)		
			1 hr	3 hr	6 hr
Compound	300	40.1	-17.6 (7.3)	-28.1 (11.8)	-33.8 (14.6)
	vehicle	40.5	-6.5 (3.0)	-13.6 (5.7)	-8.1 (3.8)
S-(+)-isomer	300	40.3	-15.0 (6.4)	-35.3 (14.8)	-40.8 (17.1)
	vehicle	38.0	-3.1 (1.8)	-6.8 (3.3)	-4.7 (2.8)

The S-isomer of 1-(2-aminopropyl)-indazol-6-ol is the preferred isomer for lowering and controlling IOP and treating glaucoma.

The Compound can be incorporated into various types of ophthalmic formulations for delivery to the eye (e.g., topically, intracamerally, or via an implant). It is preferably incorporated into topical ophthalmic formulations for delivery to the eye. The Compound may be combined with ophthalmologically acceptable preservatives, surfactants, viscosity enhancers, penetration enhancers, buffers, sodium chloride, and water to form an aqueous, sterile ophthalmic suspension or solution. Ophthalmic solution formulations may be prepared by dissolving the Compound in a physiologically acceptable isotonic aqueous buffer. Further, the ophthalmic solution may include an ophthalmologically acceptable surfactant to assist in dissolving the Compound. Furthermore, the ophthalmic solution may contain an agent to increase viscosity, such as, hydroxymethylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose, methylcellulose, polyvinylpyrrolidone, or the like, to improve the retention of the formulation in the conjunctival sac. Gelling agents can also be used, including, but not limited to, gellan and xanthan gum. In order to prepare sterile ophthalmic ointment formulations, the Compound is combined with a preservative in an appropriate vehicle, such as, mineral oil, liquid lanolin, or white petrolatum. Sterile ophthalmic gel formulations may be prepared by suspending the active ingredient in a hydrophilic base prepared from the combination of, for example, carbopol-974, or the like, according to the published formulations for analogous ophthalmic preparations; preservatives and tonicity agents can be incorporated.

The Compound is preferably formulated as a topical ophthalmic suspension or solution, with a pH of about 5 to 8. The Compound will normally be contained in these formulations in an amount 0.01% to 5% by weight, but preferably in an amount of 0.1% to 2% by weight. Thus, for topical presentation 1 to 2 drops of these formulations would be delivered to the surface of the eye 1 to 4 times per day according to the discretion of a skilled clinician.

The compounds can also be used in combination with other agents for treating glaucoma, such as, but not limited to, β-blockers (e.g., timolol, betaxolol, levobetaxolol, carteolol, levobunolol, propranolol), carbonic anhydrase inhibitors (e.g., brinzolamide and dorzolamide), α1 antagonists (e.g. nipradolol), α2 agonists (e.g., iopidine and brimonidine), miotics (e.g., pilocarpine and epinephrine), prostaglandin analogs (e.g., latanoprost, travaprost, unoprostone, and compounds set forth in U.S. Patent Nos. 5,889,052; 5,296,504; 5,422,368; and 5,151,444, "hypotensive lipids" (e.g., lumigan and compounds set forth in 5,352,708), and neuroprotectants (e.g., compounds from U.S. Patent No. 4,690,931, particularly eliprodil and R-eliprodil, as set forth in a pending application U.S.S.N. 06/203350, and appropriate compounds from WO94/13275, including memantine.

The following topical ophthalmic formulations are useful according to the present invention administered 1-4 times per day according to the discretion of a skilled clinician.

EXAMPLE 1

Ingredients	Amount (wt %)
1-(2-Aminopropyl)-indazol-6-ol (S-(+)-isomer)	0.01 – 2%
Hydroxypropyl methylcellulose	0.5%
Dibasic sodium phosphate (anhydrous)	0.2%
Sodium chloride	0.5%
Disodium EDTA (Edetate disodium)	0.01%
Polysorbate 80	0.05%
Benzalkonium chloride	0.01%
Sodium hydroxide / Hydrochloric acid	For adjusting pH to 7.3 – 7.4
Purified water	q.s. to 100%

EXAMPLE 2

Ingredients	Amount (wt %)
1-(2-Aminopropyl)-indazol-6-ol	0.01 – 2%
Methyl cellulose	4.0%
Dibasic sodium phosphate (anhydrous)	0.2%
Sodium chloride	0.5%
Disodium EDTA (Edetate disodium)	0.01%
Polysorbate 80	0.05%
Benzalkonium chloride	0.01%
Sodium hydroxide / Hydrochloric acid	For adjusting pH to 7.3 – 7.4
Purified water	q.s. to 100%

EXAMPLE 3

Ingredients	Amount (wt %)
1-(2-Aminopropyl)-indazol-6-ol (S-(+)-isomer)	0.01 – 2%
Guar gum	0.4- 6.0%
Dibasic sodium phosphate (anhydrous)	0.2%
Sodium chloride	0.5%
Disodium EDTA (Edetate disodium)	0.01%
Polysorbate 80	0.05%
Benzalkonium chloride	0.01%
Sodium hydroxide / Hydrochloric acid	For adjusting pH to 7.3 – 7.4
Purified water	q.s. to 100%

EXAMPLE 4

Ingredients	Amount (wt %)
1-(2-Aminopropyl)-indazol-6-ol	0.01 – 2%
White petrolatum and mineral oil and lanolin	Ointment consistency
Dibasic sodium phosphate (anhydrous)	0.2%
Sodium chloride	0.5%
Disodium EDTA (Edetate disodium)	0.01%
Polysorbate 80	0.05%
Benzalkonium chloride	0.01%
Sodium hydroxide / Hydrochloric acid	For adjusting pH to 7.3 – 7.4

We Claim:

1. A method for controlling intraocular pressure which comprises administering a composition comprising a pharmaceutically effective amount of 1-(2-aminopropyl)-indazol-6-ol.

2. The method of Claim 1 using the S-(+)-isomer of 1-(2-aminopropyl)-indazol-6-ol.

3. The method of Claim 1 wherein the concentration of 1-(2-aminopropyl)-indazol-6-ol is from 0.01 to 5 weight percent.

4. The method of Claim 3 wherein the concentration is 0.1 to 2 weight percent.

5. The method of Claim 2 wherein the concentration of the S-(+)-isomer of 1-(2-aminopropyl)-indazol-6-ol is from 0.01 to 5 weight percent.

6. The method of Claim 5 wherein the concentration is from 0.1 to 2 weight percent.

7. A topical ocular composition for controlling intraocular pressure comprising a pharmaceutically effective amount of 1-(2-aminopropyl)-indazol-6-ol.

8. The composition of Claim 7 using the S-(+)-isomer of 1-(2-aminopropyl)-indazol-6-ol.

9. The composition of Claim 7 wherein the concentration of 1-(2-aminopropyl)-indazol-6-ol is from 0.01 to 5 weight percent.

10. The composition of Claim 9 wherein the concentration is from 0.1 to 2 weight percent.

11. The composition of Claim 8 wherein the concentration of S-(+)-isomer of 1-(2-aminopropyl)-indazol-6-ol is from 0.01 to 5 weight percent.

12. The composition of Claim 11 wherein the concentration is from 0.1 to 2 weight percent.

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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60/190,279 17 March 2000 (17.03.2000) US

(71) Applicant (*for all designated States except US*): **ALCON UNIVERSAL LTD.** [CH/CH]; P.O. Box 62, Bosch 60, CH-6331 Hunenberg (CH).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): **COLLIER, Robert, J., Jr.** [US/US]; 3701 Big Bear Lake Drive, Arlington, TX 76016 (US). **KAPIN, Michael, A.** [US/US]; 3602 Silkwood Trail, Arlington, TX 76016 (US). **HELLBERG, Mark, R.** [US/US]; 5211 Overridge Drive, Arlington, TX 76017 (US). **DEAN, Thomas, R.** [US/US]; 101 Meadow View Court, Weatherford, TX 76087 (US).

(74) Agents: **YEAGER, Sally, S.** et al.; R & D Counsel, Mail Code Q-148, 6201 South Freeway, Fort Worth, TX 76134 (US).

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WO 01/070222 A3

(54) Title: COMPOUNDS WITH 5-HT_{1A} ACTIVITY USEFUL FOR TREATING DISORDERS OF THE OUTER RETINA

(57) Abstract: Compositions and methods for treating disorders of the outer retina with compounds with 5-HT_{1A} agonist activity are disclosed. The disorder is selected from the group consisting of: AMD; RP and other forms of heredodegenerative retinal disease; retinal detachment and tears; macular pucker; ischemia affecting the outer retina; diabetic retinopathy; damage associated with laser therapy (grid, focal, and panretinal) including photodynamic therapy (PDT); trauma; surgical (retinal translocation, subretinal surgery, or vitrectomy) or light-induced iatrogenic retinopathy; and preservation of retinal transplants. The compounds is selected from the group consisting of: tandospirone, urapidil, ziprasidone, repinotan hydrochloride, xaliproden hydrochloride (SR-57746A), buspirone, flesinoxan, EMD-68843, DU-127090, gepirone, alnespirone, PNU-95666, AP-521, flibanserin, MKC-242, lesopitron, sarizotan hydrochloride, E-5842, SUN-N4057, Org-13011, Org-12966 and 8-OH-DPAT.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/05700

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61P27/02 A61P27/06 A61K31/137 A61K31/36 A61K31/404
A61K31/428 A61K31/438 A61K31/4433 A61K31/4439 A61K31/451
A61K31/4545 A61K31/445 A61K31/4745 A61K31/496 A61K31/553

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, MEDLINE, EMBASE, BIOSIS, WPI Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	URBINA M ET AL: "5HT1A receptor agonist differentially increases cyclic AMP concentration in intact and lesioned goldfish retina. In vitro inhibition of outgrowth by forskolin." NEUROCHEMISTRY INTERNATIONAL, (1996 NOV) 29 (5) 453-60. , XP001059779 abstract page 453, column 1, paragraph 1 -page 454, column 1, paragraph 3 page 458, column 1, paragraph 2 -column 2, paragraph 2	1-3,5

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search	Date of mailing of the international search report
20 February 2002	22/04/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	A. Jakobs

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIMA L P MATUS ET AL: "Serotonin inhibits outgrowth of goldfish retina and impairs the trophic effect of taurine." JOURNAL OF NEUROSCIENCE RESEARCH, vol. 38, no. 4, 1994, pages 444-450, XP001059774 ISSN: 0360-4012 abstract	1-3,5
X	LIMA L ET AL: "8-(3H)hydroxy-2-(di-n-propylamino)tetralin binding sites in goldfish retina." NEUROCHEMICAL RESEARCH, vol. 19, no. 3, 1994, pages 249-255, XP001059781 ISSN: 0364-3190 abstract page 249, column 1 -page 250, column 1, paragraph 2 page 252, column 1, paragraph 4 -page 253, column 1, paragraph 3	1-3,5
X	WO 99 26621 A (FAHRIG THOMAS ;GERLACH IRENE (DE); HORVATH ERVIN (DE); JORK REINHA) 3 June 1999 (1999-06-03) abstract page 2, line 16 -page 5, line 16; claims 1-8	1-3,5
A	DATABASE WPI Section Ch, Week 199128 Derwent Publications Ltd., London, GB; Class B05, AN 1991-203975 XP002190916 & JP 03 128332 A (EISAI CO LTD), 31 May 1991 (1991-05-31) abstract	1-6
A	CHU T-C ET AL: "8OH-DPAT-INDUCED OCULAR HYPOTENSION: SITES AND MECHANISMS OF ACTION" EXPERIMENTAL EYE RESEARCH, ACADEMIC PRESS LTD., LONDON, GB, vol. 69, no. 2, August 1999 (1999-08), pages 227-238, XP000879248 ISSN: 0014-4835 abstract	1-6

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/05700

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE WPI Section Ch, Week 199049 Derwent Publications Ltd., London, GB; Class B03, AN 1990-364832 XP002190917 & JP 02 262518 A (KAKEN PHARM CO LTD), 25 October 1990 (1990-10-25) abstract ---	1-6
A	CHIDLOW G ET AL: "THE 5-HT1A RECEPTOR AGONIST 8-OH-DPAT LOWERS INTRAOCULAR PRESSURE IN NORMOTENSIVE NZW RABBITS" EXPERIMENTAL EYE RESEARCH, ACADEMIC PRESS LTD., LONDON, GB, vol. 69, no. 6, 1999, pages 587-593, XP000878788 ISSN: 0014-4835 abstract ---	1-6
A	WO 98 18458 A (OSBORNE NEVILLE ;DESANTIS LOUIS JR (US); ALCON LAB INC (US); SALLE) 7 May 1998 (1998-05-07) the whole document ----	1-6

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1,3,4,6 relate to a compound/therapeutic application defined by reference to a desirable characteristic or property, namely a compound with 5-HT1A agonist activity.
The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound/therapeutic application by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds specifically mentioned in claims 3 and 5 in relation to the diseases specified in claims 3,4,6.

Remark: the abreviations used in the claims should be replaced by the full words.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/05700

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
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			AT	206615	T	15-10-2001
			AU	1668599	A	15-06-1999
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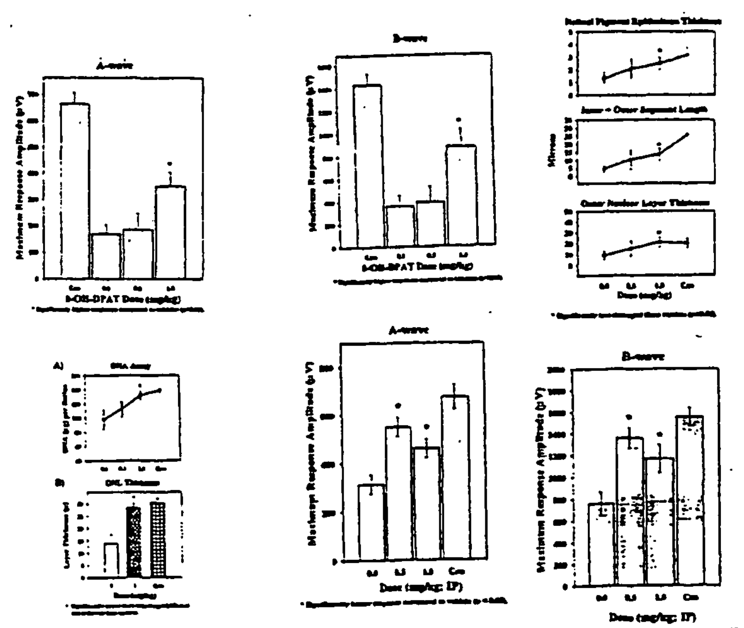

(57) Abstract: Compositions and methods for treating disorders of the outer retina with compounds with 5-HT_{1A} agonist activity are disclosed.

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COMPOUNDS WITH 5-HT$_{1A}$ ACTIVITY USEFUL FOR TREATING DISORDERS OF THE OUTER RETINA

The present invention is directed to compounds with 5-HT$_{1A}$ agonist activity useful for treating disorders of the outer retina resulting from acute or chronic degenerative conditions or diseases of the eye.

Background of the Invention

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly, with an incidence of about 20% in adults 65 years of age increasing to 37% in individuals 75 years or older. Non-exudative AMD is characterized by drusen accumulation and atrophy of rod and cone photoreceptors in the outer retina, retinal pigment epithelium (RPE), Bruch's membrane and choriocapillaris; while exudative AMD leads to choroidal neovascularization (Green and Enger, *Ophthalmol*, 100:1519-35, 1993; Green et al., *Ophthalmol*, 92:615-27, 1985; Green and Key, *Trans Am Ophthalmol Soc*, 75:180-254, 1977; Bressler et al., *Retina*, 14:130-42, 1994; Schneider et al., *Retina*, 18:242-50, 1998; Green and Kuchle (1997). In: Yannuzzi, L.A., Flower, R.W., Slakter, J.S. (Eds.) *Indocyanine green angiography*. St. Louis: Mosby, p. 151-6). Retinitis pigmentosa (RP) represents a group of hereditary dystrophies characterized by rod degeneration with secondary atrophy of cone photoreceptors and underlying pigment epithelium. (Pruett, *Trans Am Ophthalmol Soc*, 81:693-735, 1983; Heckenlively, *Trans Am Ophthalmol Soc*, 85:438-470, 1987; Pagon, *Sur Ophthalmol*, 33:137-177, 1988; Berson, *Invest Ophthalmol Vis Sci*, 34:1659-1676, 1993; Nickells and Zack, *Ophthalmic Genet*, 17:145-65, 1996). The pathogenesis of retinal degenerative diseases such as AMD and RP is multifaceted and can be triggered by environmental factors in normal individuals or in those who are genetically predisposed. To date more than 100 genes have been mapped or cloned that may be associated with various outer retinal degenerations.

Light exposure is an environmental factor that has been identified as a contributing factor to the progression of retinal degenerative disorders such as AMD (Young, *Sur Ophthal*, 32:252-269, 1988; Taylor, et al., *Arch Ophthal*, 110:99-104, 1992; Cruickshank, et al., *Arch Ophthal*, 111:514-518, 1993). Photo-oxidative stress leading to light damage to retinal cells has been shown to be a useful model for studying retinal degenerative diseases for the following reasons: damage is primarily to the photoreceptors and retinal pigment epithelium (RPE) of the outer retina, the

same cells that are affected in heredodegenerative diseases (Noell et al., *Invest Ophthal Vis Sci*, 5, 450-472, 1966; Bressler et al., *Sur Ophthal*, 32, 375-413, 1988; Curcio et al., *Invest Ophthal Vis Sci*, 37, 1236-1249, 1996); apoptosis is the cell death mechanism by which photoreceptor and RPE cells are lost in AMD and RP, as well as following a photo-oxidative induced cell injury (Ge-Zhi et al., *Trans AM Ophthal Soc*, 94, 411-430, 1996; Abler et al., *Res Commun Mol Pathol Pharmacol*, 92, 177-189, 1996; Nickells and Zack, *Ophthalmic Genet*, 17:145-65, 1996); light has been implicated as an environmental risk factor for progression of AMD and RP (Taylor et al., *Arch Ophthalmol*, 110, 99-104, 1992; Naash et al., *Invest Ophthal Vis Sci*, 37, 775-782, 1996); and therapeutic interventions which inhibit photo-oxidative injury have also been shown to be effective in animal models of heredodegenerative retinal disease (LaVail et al., *Proc Nat Acad Sci*, 89, 11249-11253, 1992; Fakforovich et al., *Nature*, 347, 83-86, 1990; Frasson et al., *Nat. Med.* 5, 1183-1187, 1990).

A number of different compound classes have been identified in various animal models that minimize retinal photo-oxidative injury. They include: antioxidants such as ascorbate (Organisciak et al., *Invest Ophthal Vis Sci*, 26:1589-1598, 1985), dimethylthiourea (Organisciak et al., *Invest Ophthal Vis Sci*, 33:1599-1609, 1992; Lam et al., *Arch Ophthal*, 108:1751-1752, 1990), α-tocopherol (Kozaki et al., *Nippon Ganka Gakkai Zasshi*, 98:948-954, 1994) and β-carotene (Rapp et al., *Cur Eye Res*, 15:219-232, 1995); calcium antagonists such as flunarizine (Li et al., *Exp Eye Res*, 56: 71-78, 1993; Edward et al., *Arch Ophthal*, 109, 554-622, 1992; Collier et al., *Invest Ophthal Vis Sci*, 36:S516); growth factors such as basic-fibroblast growth factor, brain derived nerve factor, ciliary neurotrophic factor, and interleukin-1-β (LaVail et al., *Proc Nat Acad Sci*, 89, 11249-11253, 1992); glucocorticoids such as methylprednisolone (Lam et al., *Graefes Arch Clin Exp Ophthal, 231*, 729-736, 1993) and dexamethasone (Fu et al., *Exp Eye Res*, 54, 583-594, 1992); iron chelators such as desferrioxamine (Li et al., *Cur Eye Res*, 2, 133-144, 1991); NMDA-antagonists such as eliprodil and MK-801 (Collier et al., *Invest Ophthal Vis Sci*, 40:S159, 1999).

Serotonergic 5-HT_{1A} agonists (i.e., buspirone, ziprasidone, urapidil) have either been registered or launched for the treatment of anxiety, hypertension, schidzophrenia, psychosis or depression-bipolar disorders. In addition, 5-HT_{1A} agonists have been shown to be neuroprotective in various animal models and are being evaluated in the clinic to treat cerebral ischemia, head trauma, Alzheimer's Disease, Multiple Sclerosis and amytrophic lateral sclerosis. The 5-HT_{1A} agonists, 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin) and ipsapirone, were shown to

prevent NMDA-induced excitotoxic neuronal damage in the rat magnocellular nucleus basalis (Oosterink et al., *Eur J Pharmacol*, 358:147-52, 1998), dosing with Bay-x-3702 significantly reduced ischemic damage in a rat acute subdermal hematoma model (Alessandri et al., *Brain Res*, 845:232-5, 1999), while 8-OH-DPAT, Bay-x-3702, urapidil, gepirone and CM 57493 significantly reduced cortical infarct volume in the rat (Bielenberg and Burkhardt, *Stroke*, 21(Suppl): IV161-3; Semkova et al., *Eur J Pharmacol*, 359:251-60, 1998; Peruche et al., *J Neural Transm – Park Dis Dement Sect*, 8:73-83, 1994) and mouse (Prehn et al., *Eur J Pharmacol*, 203:213-22, 1991; Prehn et al., *Brain Res*, 630:10-20, 1993) after occlusion of the middle cerebral artery. In addition, treatment of rats with SR 57746A, a potent 5-HT_{1A} agonist, has been shown to be neuroprotective following 4-vessel transient global ischemia, vincristine sulphate induced septohippocampal lesions, acrylamide-induced peripheral neuropathy, and sciatic nerve crush (Fournier et al., *Neurosci*, 55:629-41, 1993) and has been shown to delay the progression of motor neuron degeneration in *pmn* mice (Fournier et al., *Br J Pharmacol*, 124:811-7, 1998).

This class of compounds has been disclosed for the treatment of glaucoma (lowering and controlling IOP), see e.g., WO 98/18458 (DeSantis, et al) and EP 0771563A2 (Mano, et al.). Osborne, et al. (Ophthalmologica, Vol. 210:308-314, 1996) teach that 8-hydroxydipropylaminotetralin (8-OH-DPAT) (a 5-HT_{1A} agonist) reduces IOP in rabbits. Wang, et al. (Current Eye Research, Vol. 16(8):769-775, August 1997, and IVOS, Vol. 39(4), S488, March, 1998) disclose that 5-methylurapidil, an α_{1A} antagonist and 5-HT_{1A} agonist lowers IOP in the monkey, but due to its α_{1A} receptor activity. Also, 5-HT_{1A} <u>antagonists</u> are disclosed as being useful for the treatment of glaucoma (elevated IOP) (e.g. WO 92/0338, McLees). Furthermore, DeSai, et al. (WO 97/35579) and Macor, et al. (U.S. 5,578,612) disclose the use of 5-HT_1 and $5\text{-HT}_{1\text{-like}}$ agonists for the treatment of glaucoma (elevated IOP). These anti-migraine compounds are $5\text{-HT}_{1B,D,E,F}$ agonists, e.g., sumatriptan and naratriptan and related compounds.

<u>Brief Description of the Drawings</u>

<u>Figures 1A and 1B</u> show the preservation of the ERG a- and b-wave function in rats dosed systemically with 8-OH-DPAT and exposed to a severe photo-oxidative insult.

<u>Figure 2</u> shows protection of retinal morphology (photoreceptors and RPE) in rats dosed systemically with 8-OH-DPAT and exposed to a severe photo-oxidative insult.

Figure 3 shows protection of retinal DNA, a measure of retinal cell number (A), and complete protection of retinal morphology (photoreceptors) in rats dosed systemically with buspirone and exposed to a severe photo-oxidative insult.

Figure 4A and 4B show the preservation of the ERG a- and b-wave function in rats dosed systemically with SR-57746A and exposed to a severe photo-oxidative insult.

Summary of the Invention

The present invention is directed to 5–HT$_{1A}$ agonists which have been discovered to be useful in treating disorders of the outer retina, particularly: AMD; RP and other forms of heredodegenerative retinal disease; retinal detachment and tears; macular pucker; ischemia affecting the outer retina; diabetic retinopathy; damage associated with laser therapy (grid, focal, and panretinal) including photodynamic therapy (PDT); trauma; surgical (retinal translocation, subretinal surgery, or vitrectomy) or light-induced iatrogenic retinopathy; and preservation of retinal transplants.

Description of Preferred Embodiments

Serotonergic 5-HT$_{1A}$ agonists have been shown to be potent neuroprotective agents following varying insults to the central nervous system. Unexpectedly, we have demonstrated that 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin), buspirone and SR-57746A exhibit potent neuroprotective activity in the retina and prevent light-induced apoptotic cell death to photoreceptors and RPE cells. We have found that treatment with buspirone can completely prevent photo-oxidative induced retinopathy and significantly reduce loss of retinal DNA and ONL thinning. The safety advantages of some of these compounds make them particularly desirable for both acute and chronic therapies. Such an agent would have utility in the treatment of various outer retinal degenerative diseases.

In our light damage paradigms, antioxidants were either ineffective (α-tocopherol) or marginally effective at high doses (ascorbate, vitamin E analogs). Similarly, some calcium antagonists (flunarizine, nicardipine) were moderately effective while others (nifedipine, nimodipine, verapamil) had no effect in preventing light-induced functional or morphological changes. However, it has been discovered that 5-HT$_{1A}$ agonists are 100-fold more potent in these light damage paradigms and therefore are useful for treating disorders of the outer retina.

The invention contemplates the use of any pharmaceutically acceptable 5-HT_{1A} agonist, including pharmaceutically acceptable salts, for treating disorders of the outer retina (Compounds). Pharmaceutically acceptable means the Compounds can be safely used for the treatment of diseases of the outer retina. As used herein, the outer retina includes the RPE, photoreceptors, Muller cells (to the extent that their processes extend into the outer retina), and the outer plexiform layer. The compounds are formulated for systemic or local ocular delivery.

Disorders of the outer retina encompass acute and chronic environmentally induced (trauma, ischemia, photo-oxidative stress) degenerative conditions of the photoreceptors and RPE cells in normal or genetically predisposed individuals. This would include, but not limited to, AMD, RP and other forms of heredodegenerative retinal disease, retinal detachment, tears, macular pucker, ischemia affecting the outer retina, diabetic retinopathy, damage associated with laser therapy (grid, focal and panretinal) including photodynamic therapy (PDT), thermal or cryotherapy, trauma, surgical (retinal translocation, subretinal surgery or vitrectomy) or light induced iatrogenic retinopathy and preservation of retinal transplants.

Compounds of the present invention have potent affinity for 5-HT_{1A} receptors with IC_{50} values that range up to about 500 nM (preferably less than 100 nM). These Compounds are also either full or partial agonists with IC_{50} values ranging up to about 1 μM (preferably less than 500 nM). Representative 5-HT_{1A} agonists useful according to the present invention include, but are not limited to: tandospirone, urapidil, ziprasidone, repinotan hydrochloride, xaliproden hydrochloride (SR-57746A), buspirone, flesinoxan, EMD-68843, DU-127090, gepirone, alnespirone, PNU-95666, AP-521, flibanserin, MKC-242, lesopitron, sarizotan hydrochloride, Org-13011, Org-12966, E-5842, SUN-N4057, and 8-OH-DPAT.

Receptor binding and agonist activity according to this invention can be determined using the following methods.

METHOD 1

5-HT_{1A} Receptor Binding Assay

5-HT_{1A} binding studies were performed with human cloned receptors expressed in Chinese hamster ovary (CHO) cells using (^{3}H)8-OH DPAT as the ligand. Membranes from Chinese hamster ovary cells (CHO) expressing cloned 5-HT_{1A} receptors (manufactured for NEN by Biosignal, Inc., Montreal, Canada) were homogenized in approximately 40 volumes of 50 mM Tris pH 7.4 for 5 sec. Drug dilutions were made using a Beckman Biomek 2000 robot (Beckman Instruments, Fullerton, CA). Incubations were conducted with membrane prep, test compounds, and 0.25 nM [^{3}H]8-OH-DPAT (NEN, Boston, MA) in the same buffer at 27°C for 1 h. Assays were terminated by rapid vacuum filtration over Whatman GF/B glass fiber filters pre-soaked in 0.3% polyethyleneimine. Bound radioactivity was measured using liquid scintillation spectrometry. Data were analyzed using non-linear curve fitting programs (Sharif et al., J Pharmac Pharmacol, 51: 685-694, 1999).

Ligand binding studies can also be run using membrane preparations from calf and rat brain (local source) and human cortex membranes. Specific brain regions were dissected out, homogenized in 10 volumes of 0.32 M sucrose and centrifuged for 10 min at 700 x g. The resulting supernatant was centrifuged at 43,500 x g for 10 min and the pellet re-suspended in 50 mM Tris-HCl (pH 7.7, 25°C) using a 10 sec polytron treatment. Aliquots were stored at –140° C. To remove endogenous serotonin, the preps were incubated at 37° C for 10 min prior to the experiment. Assay incubations were terminated by rapid filtration over Whatman GF/C filters using a Brandel cell harvester. K_i values were calculated using the Cheng-Prusoff equation (De Vry et al., J Pharm Exper Ther, 284:1082-1094, 1998.)

METHOD 2

5-HT_{1A} Functional Assays

The function of Compounds of the present invention can be determined using a variety of methods to assess the functional activity of 5-HT_{1A} agonists. One such assay is performed using hippocampal slices from male Sprague-Dawley rats, measuring the inhibition of forskolin-stimated adenylate cyclase (J Med Chem, 42:36, 1999; J Neurochem, 56:1114, 1991; J Pharm Exper Ther, 284:1082, 1998). Rat hippocampal membranes were homogenized in 25 volumes of 0.3 M sucrose containing 1mM EGTA, 5 mM EDTA, 5 mM dithiothreitol, and 20 mM Tris-HCl, pH 7.4 at 25°C. The homogenate was centrifuged for 10 m in at 1,000 x g. The

supernatant subsequently was centrifuged at 39,000 x g for 10 min. The resulting pellet was re-suspended in homogenization buffer at a protein concentration of approximately 1 mg/ml and aliquots were stored at −140°C. Prior to use, the membranes were rehomogenized in a Potter-Elvehjem homogenizer. Fifty μl of the membrane suspension (50 μg protein) were added to an incubation buffer containing 100 mM NaCl, 2 mM magnesium acetate, 0.2 mM ATP, 1 mM cAMP, 0.01 mM GTP, 0.01 mM forskolin, 80 mM Tris-HCl, 5 mM creatine phosphate, 0.8 U/μl creatine phosphokinase, 0.1 mM IBMX, 1-2 μCi α-[^{32}P]ATP. Incubations with test compounds (10 min at 30°C) were initiated by the addition of the membrane solution to the incubation mixture (prewarmed 5 min at 30°C). [^{32}P]cAMP was measured according to the method of Salomon (Adv Cyclic Nucleotide Res, 10:35-55, 1979). Protein was measured using the Bradford (Anal Biochem, 72:248-254, 1976) assay.

Functional activity can also be determined in recombinant human receptors according to the method of Schoeffter et al. (Neuropharm, 36:429-437, 1997). HeLa cells transfected with recombinant human 5-HT_{1A} receptors were grown to confluence in 24-well plates. The cells were rinsed with 1 ml of Hepes-buffered saline (in mM) NaCl 130, KCl 5.4, $CaCl_2$ 1.8, $MgSO_4$ 0.8, NaH_2PO_4 0.9, glucose 25, Hepes 20, pH 7.4, and phenol red 5 mg/l. The cells were labelled with 6 μCi/ml of [^{3}H]adenine (23 Ci/mmol, Amersham, Rahn AG, Zurich, Switzerland) in 0.5 ml of saline at 37 °C for 2 hr. The plates were subsequently rinsed twice with 1 ml of buffered saline containing 1mM isobutylmethylxanthine. The cells were incubated for 15 min in 1 ml of this solution (37 °C) in the presence or absence of 10 μM forskolin and the test compound. The buffer was then removed and 1 ml of 5% trichloroacetic acid (TCA) containing 0.1 mM cAMP and 0.1 mM ATP was added to extract the samples. After 30 min at 4°C, the TCA extracts were subjected to chromatographic separation on Dowex AG 50W-X4 and alumina columns (Salomon, Meth Enzymol, 195: 22-28, 1991). Cyclic AMP production was calculated as the ratio [^{3}H]cAMP/([^{3}H]cAMP + [^{3}H]ATP).

The above procedures described in Methods 1 and 2 were used to generate the following data.

Table 1. 5-HT_{1A} Receptor Binding and Functional Assay Data.

Compound	Receptor Binding (IC_{50} nM, SEM)	cAMP Inhibition (EC_{50})
(R,S) 8-OH-DPAT	1.5 nM	4.7 nM
(R) 8-OH-DPAT	0.5 nM	2.6 nM
SR-57746A	2.5 nM	3.7 nM

In general, for degenerative diseases, the 5-HT_{1A} agonists of this invention are administered orally with daily dosage of these compounds ranging between about 0.001 and about 500 milligrams. The preferred total daily dose ranges between about 1 and about 100 milligrams. Non-oral administration, such as, intravitreal, topical ocular, transdermal patch, subdermal, parenteral, intraocular, subconjunctival, or retrobulbar or subtenon's injection, trans scleral (including iontophoresis), or slow release biodegradable polymers or liposomes may require an adjustment of the total daily dose necessary to provide a therapeutically effective amount of the compound. The 5-HT_{1A} agonists can also be delivered in ocular irrigating solutions. Concentrations should range from about 0.001 μM to about 100 μM, preferably about 0.01 μM to about 5 μM.

The 5-HT_{1A} agonists can be incorporated into various types of ophthalmic formulations for delivery to the eye (e.g., topically, intracamerally, or via an implant). They may be combined with ophthalmologically acceptable preservatives, surfactants, viscosity enhancers, gelling agents, penetration enhancers, buffers, sodium chloride, and water to form aqueous, sterile ophthalmic suspensions or solutions or preformed gels or gels formed in situ. Ophthalmic solution formulations may be prepared by dissolving the compound in a physiologically acceptable isotonic aqueous buffer. Further, the ophthalmic solution may include an ophthalmologically acceptable surfactant to assist in dissolving the compound. The ophthalmic solutions may contain a viscosity enhancer, such as, hydroxymethylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose, methylcellulose, polyvinyl-pyrrolidone, or the like, to improve the retention of the formulation in the conjunctival sac. In order to prepare sterile ophthalmic ointment formulations, the active ingredient is combined with a preservative in an appropriate vehicle, such as, mineral oil, liquid lanolin, or white petrolatum. Sterile ophthalmic gel formulations may be prepared by suspending the

active ingredient in a hydrophilic base prepared from the combination of, for example, carbopol-940, or the like, according to the published formulations for analogous ophthalmic preparations; preservatives and tonicity agents can be incorporated.

If dosed topically, the 5-HT_{1A} agonists are preferably formulated as topical ophthalmic suspensions or solutions, with a pH of about 4 to 8. The 5-HT_{1A} agonists will normally be contained in these formulations in an amount .001% to 5% by weight, but preferably in an amount of .01% to 2% by weight. Thus, for topical presentation, 1 to 2 drops of these formulations would be delivered to the surface of the eye 1 to 4 times per day according to the discretion of a skilled clinician.

The following topical ophthalmaic formulations are useful according to the present invention administered 1-4 times per day according to the discretion of a skilled clinician.

EXAMPLE 1

Ingredients	Amount (wt %)
Buspirone	0.01 – 2%
Hydroxypropyl methylcellulose	0.5%
Dibasic sodium phosphate (anhydrous)	0.2%
Sodium chloride	0.5%
Disodium EDTA (Edetate disodium)	0.01%
Polysorbate 80	0.05%
Benzalkonium chloride	0.01%
Sodium hydroxide / Hydrochloric acid	For adjusting pH to 7.3 – 7.4
Purified water	q.s. to 100%

EXAMPLE 2

Ingredients	Amount (wt %)
Buspirone	0.01 – 2%
Methyl cellulose	4.0%
Dibasic sodium phosphate (anhydrous)	0.2%
Sodium chloride	0.5%
Disodium EDTA (Edetate disodium)	0.01%
Polysorbate 80	0.05%
Benzalkonium chloride	0.01%
Sodium hydroxide / Hydrochloric acid	For adjusting pH to 7.3 – 7.4
Purified water	q.s. to 100%

EXAMPLE 3

Ingredients	Amount (wt %)
Compound	0.01 – 2%
Guar gum	0.4- 6.0%
Dibasic sodium phosphate (anhydrous)	0.2%
Sodium chloride	0.5%
Disodium EDTA (Edetate disodium)	0.01%
Polysorbate 80	0.05%
Benzalkonium chloride	0.01%
Sodium hydroxide / Hydrochloric acid	For adjusting pH to 7.3 – 7.4
Purified water	q.s. to 100%

EXAMPLE 4

Ingredients	Amount (wt %)
Xaliproden hydrochloride	0.01 – 2%
White petrolatum and mineral oil and lanolin	Ointment consistency
Dibasic sodium phosphate (anhydrous)	0.2%
Sodium chloride	0.5%
Disodium EDTA (Edetate disodium)	0.01%
Polysorbate 80	0.05%
Benzalkonium chloride	0.01%
Sodium hydroxide / Hydrochloric acid	For adjusting pH to 7.3 – 7.4

EXAMPLE 5

10mM IV Solution w/v%	
Buspirone	0.384%
L-Tartaric acid	2.31%
Sodium hydroxide	pH 3.8
Hydrochloric acid	pH 3.8
Purified water	q.s. 100%

EXAMPLE 6

5mg Capsules	
Ingredient	**mg/capsule (Total Wt. 22a mg)**
Buspirone Hydrochloride	5
Lactose, anhydrous	55.7
Strach, Sodium carboxy-methyl	8
Cellulose, microcrystalline	30
Colloidal silicon dioxide	.5
Magnesium sterage	.8

METHOD 3

Neuroprotective effects in the rat photo-oxidative induced retinopathy model

The retinal protective effect of these 5-HT_{1A} agonists were evaluated in our photo-oxidative induced retinopathy paradigm.

Induction of Photochemical Lesion. Photochemical lesions were induced in dark adapted rats (24 hour) by exposure to (220 fc) blue light (half-amplitude bandpass = 435-475 nm) for 6 hours. Animals were allowed to recover for 5 days in darkness prior to electrodiagnostic evaluation of retinal function. Rats were single housed in clear polycarbonate cages during this light exposure.

Electrodiagnostic Evaluation. The electroretinogram (ERG) was recorded from anesthetized rats after a 24-hour dark-adaptation period. Rats were anesthetized by IP injection with Ketamine-HCl (75 mg/Kg) and Xylazine (6 mg/Kg). Flash ERGs recorded from a platinum-iridium wire loop electrode positioned on the cornea were elicited by viewing a ganzfeld. Electrical responses to a series of light flashes increasing in intensity were digitized to analyze temporal characteristics of the waveform and response voltage-log intensity (VlogI) relationship. Changes in the ERG a-wave are associated with photoreceptor and retinal pigment epithelium damage while damage to the inner retina is reflected in changes in the ERG b-wave.

Assessment of Retinal Morphology. Ocular tissues were obtained from control and drug or vehicle dosed rats and fixed by immersion into a mixture of 2%

paraformaldehyde and 2% glutaraldehyde. Fixed eyeballs were dehydrated in an ascending ethanol series, embedded in JB-4 plastic resin, and 1 to 1.5-micron thick sections were analyzed using a quantitative computer image analysis system attached to the microscope. Retinal layer thickness (retinal pigment epithelium, RPE; outer nuclear layer thickness, ONL; inner nuclear layer thickness, INL; and length of photoreceptor inner and outer segments, IS+OS) was measured.

Assessment of DNA Changes. Albino rats were euthanized by CO_2 inhalation and individual retinas were frozen in separate tubes. Each retina had been sonicated in 0.8 ml (2.0 M NaCl, 50 mM $NaPO_4$, pH 7.4, 2 mM EDTA) to yield a uniform homogenate, and stored frozen. Aliquots (0.1 ml) of each sample were diluted 10-fold with 2.0 M NaCl, 50 mM $NaPO_4$. pH 7.4, 2 mM EDTA containing 1.1 μg/ml bisbenzimidazole (Hoechst 33258). A standard curve was constructed using calf thymus DNA from 0 to 25 μg/ml in the same buffer. Triplicate 0.2 ml aliquots of each retina sample and standard were pipetted into a 96 well plate for fluorescence measurements in the Cytofluor II. The excitation wavelength was 360 nm, and the emission wavelength was 460 nm.

Subjects and Dosing. Male Sprague Dawley rats were randomly assigned to drug and vehicle experimental groups. Control rats were housed in their home cage under normal cyclic light exposure. All rats were dosed 48, 24 and 0 hours prior to a 6-hour blue-light exposure. Dosing was as follows:

1.) ***8-OH-DPAT*** **(8-hydroxy-2-(di-n-propylamino)tetralin):** Rats receiving either vehicle (N=10) or 8-OH-DPAT (0.5 mg/kg [N=5] or 1.0 mg/kg [N=10]) were given three subcutaneous (SC) injections prior to light exposure. Five rats were used as controls. Retinal protection was assessed by analyzing the ERG response and measuring changes in retinal morphology.

2.) ***Buspirone***: For DNA quantitation, six rats per treatment group were dosed IP with vehicle or buspirone (0.5 and 1 mg/kg) prior to light exposure. Retinas from seven normal rats were used as controls. To evaluate changes in retinal morphology, rats were dosed (IP) with either vehicle (N=8) or buspirone (1.0 mg/kg [N=9]). Six rats were used as controls. Retinal protection was assessed by quantitating changes in retinal DNA and measuring changes in retinal morphology.

3.) ***SR-57746A***: Rats were dosed (IP) with vehicle (N=15) or SR-57746A (0.5 mg/kg [N=5] or 1 mg/kg [N=15]). Eleven rats were used as controls. The ERG was analyzed after a 5-day recovery period to assess retinal protection.

8-OH-DPAT Evaluation Results. Blue-light exposure for 6 hours resulted in a significant diminution of the ERG response amplitude (ANOVA, $p < 0.001$; Bonferroni t-test, $p < 0.05$) compared to normals when measured after a 5-day recovery period (Figures 1A and B). Blue-light exposure resulted in a 75% reduction in the maximum a- and b-wave amplitudes in vehicle dosed rats compared to controls. In addition, threshold responses were lower and evoked at brighter flash intensities.

Rats dosed with 8-OH-DPAT showed dose-dependent protection of outer and inner retina function against this photo-oxidative induced retinopathy (Figures 1A and B). Maximum a- and b-wave response amplitudes in 8-OH-DPAT (0.5 mg/kg) dosed rats were not different than vehicle dosed rats and were approximately 27% of control amplitudes. However, maximum a- and b-wave response amplitudes from 8-OH-DPAT (1.0 mg/kg) dosed rats were approximately 53% and 61% of normal, respectively, and significantly higher than responses measured in vehicle dosed rats (Figures 1A and 1B).

Consistent with these ERG changes, morphometric analysis of these retinas after a 3-week recovery period demonstrated a significant (ANOVA, $p<0.01$) loss of photoreceptor cells, shortening of photoreceptor inner + outer segment length, and flattening of the RPE in vehicle dosed animals. No significant changes in the thickness of the INL were detected. ONL thickness was reduced 73%, inner + outer segment length was reduced 82%, and RPE thickness was reduced 59% compared to controls (Figure 2). Lesions observed in rats dosed with 8-OH-DPAT (0.5 mg/kg) were not significantly different than lesions measured in vehicle dosed rats. While ERGs were reduced approximately 63%, the ONL thickness was reduced by 53%, photoreceptor segment length was reduced 60%, and the RPE thickness was reduced 34%. However, photic lesions observed in rats dosed with 8-OH-DPAT (1.0 mg/kg) were significantly different from vehicle dosed rats. While ERG response amplitudes were greater than 50% of normal, the ONL thickness was 2.4 fold thicker, photoreceptor segment length was 2.9 times longer, and RPE thickness was 1.9 times thicker compared to vehicle dosed rats.

Buspirone Evaluation Results. As seen in Figure 3A, vehicle dosed retinal DNA levels were significantly reduced (ANOVA, $p=0.017$) about 30% from control levels.

No significant differences were measured between groups dosed with vehicle or 0.1 mg/kg buspirone. Retinal protection was measured in rats dosed with buspirone (1 mg/kg). Retinal DNA levels were significantly higher than measured in vehicle dosed rats, but not significantly different than controls.

Blue-light exposure for 6 hours resulted in a significant reduction in photoreceptor number (ANOVA, $p<0.05$). Morphometric analysis of these retinas after a 4-week recovery period demonstrated a 54% thinning of the outer nuclear layer in vehicle dosed rats compared to controls (Figure 3B). However, no significant difference in ONL thickness was measured between normal and buspirone treated rats. In rats dosed with buspirone (1 mg/kg) the ONL thickness was 28.3 μ compared to 30.4 μ in normal rats.

__SR-57746A Evaluation Results__. Significant protection of retinal function was measured in light-exposed rats dosed with SR-57746A (0.5 and 1.0 mg/kg). Maximum a- and b-wave response amplitudes were reduced by 50% in vehicle dosed rats compared to controls (Figures 4A and B). Maximum responses were 82% of controls in rats dosed with SR-57746A (0.5 mg/kg) and 70% of normal in rats dosed with 1 mg/kg.

Conclusion. These 5-HT_{1A} agonists (8-OH-DPAT, buspirone, and SR-57746A) demonstrated good potency and efficacy in this oxidative model of retinal degenerative disease. Functional and structural protection were achieved in rats dosed on three consecutive days with a dose as low as 1 mg/kg.

We Claim:

1. A method for treating disorders of the outer retina which comprises administering a pharmaceutically effective amount of a compound with 5-HT_{1A} agonist activity.

2. The method of Claim 1, wherein the compound is selected from the group consisting of: tandospirone, urapidil, ziprasidone, repinotan hydrochloride, xaliproden hydrochloride (SR-57746A), buspirone, flesinoxan, EMD-68843, DU-127090, gepirone, alnespirone, PNU-95666, AP-521, flibanserin, MKC-242, lesopitron, sarizotan hydrochloride, E-5842, SUN-N4057, Org-13011, Org-12966 and 8-OH-DPAT.

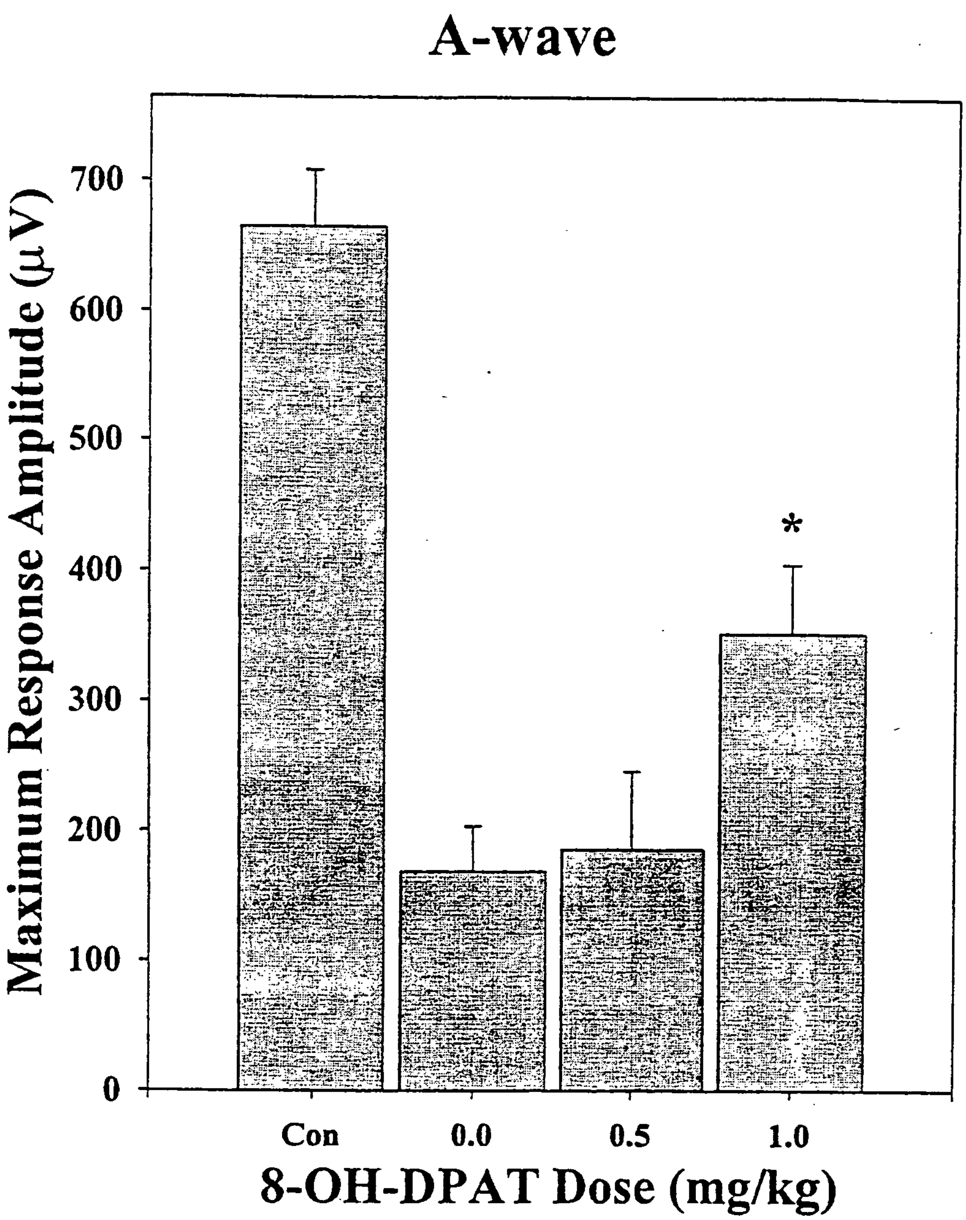
3. The method of Claim 1 wherein the disorder is selected from the group consisting of: AMD; RP and other forms of heredodegenerative retinal disease; retinal detachment and tears; macular pucker; ischemia affecting the outer retina; diabetic retinopathy; damage associated with laser therapy (grid, focal, and panretinal) including photodynamic therapy (PDT); trauma; surgical (retinal translocation, subretinal surgery, or vitrectomy) or light-induced iatrogenic retinopathy; and preservation of retinal transplants.

4. The method of Claim 3 wherein the disorder is AMD.

5. The method of Claim 3 wherein the compound is selected from the group consisting of: tandospirone, urapidil, ziprasidone, repinotan hydrochloride, xaliproden hydrochloride (SR-57746A), buspirone, flesinoxan, EMD-68843, DU-127090, gepirone, alnespirone, PNU-95666, AP-521, flibanserin, MKC-242, lesopitron, sarizotan hydrochloride, E-5842, SUN-N4057, Org-13011, Org-12966 and 8-OH-DPAT.

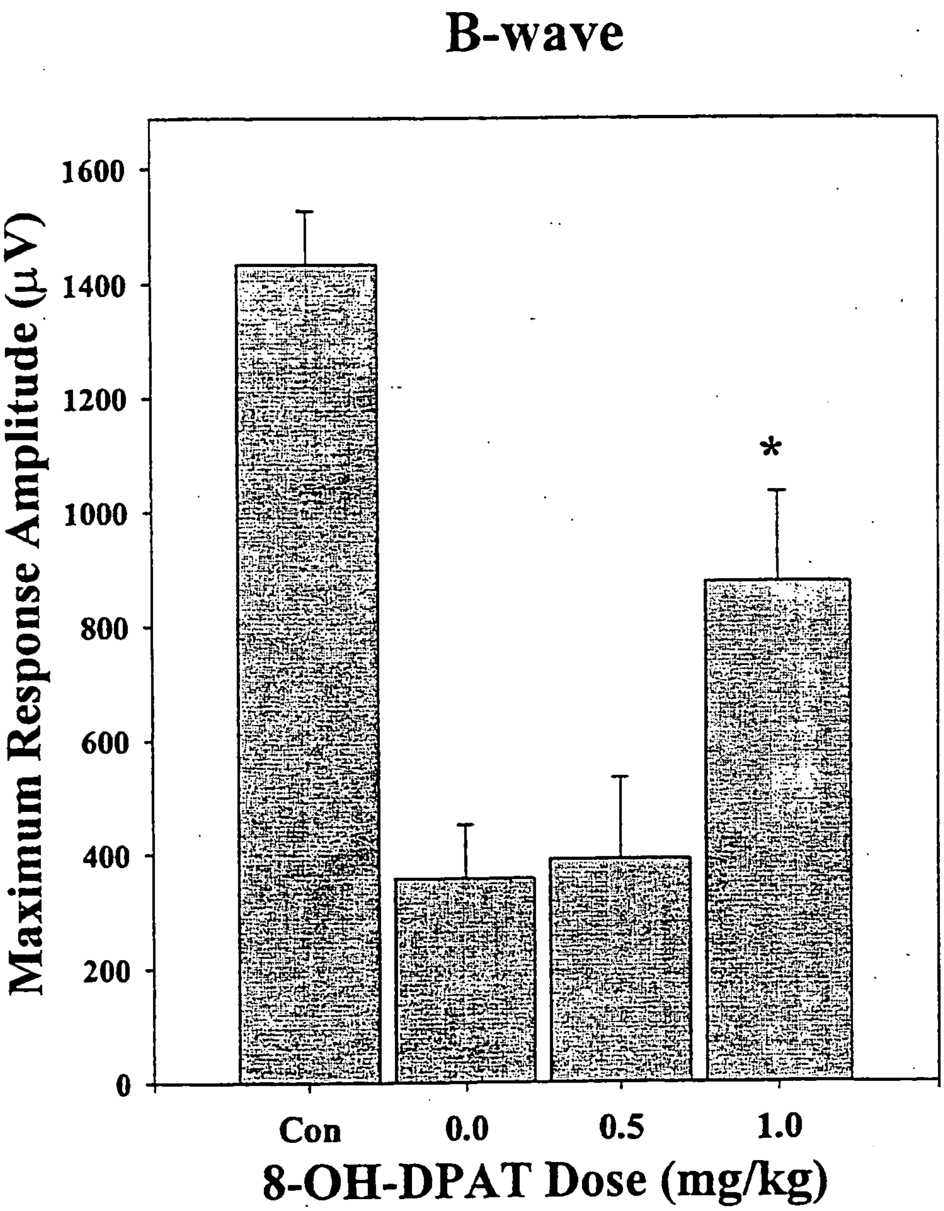
6. The method of Claim 5 wherein the disorder is selected from the group consisting of AMD, RP, and diabetic retinopathy.

FIGURE 1A



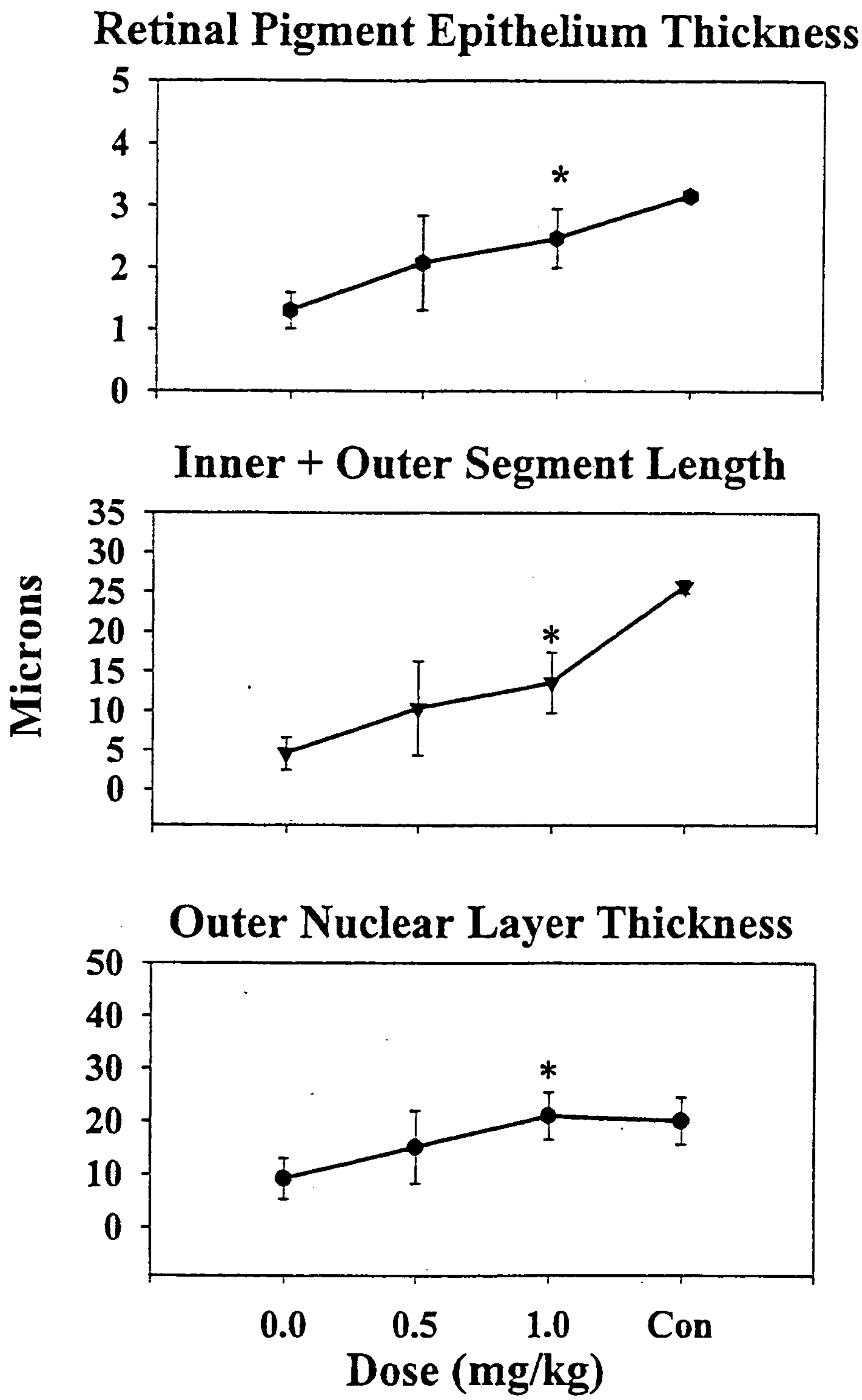
* Significantly higher responses compared to vehicles (p<0.05).

FIGURE 1B



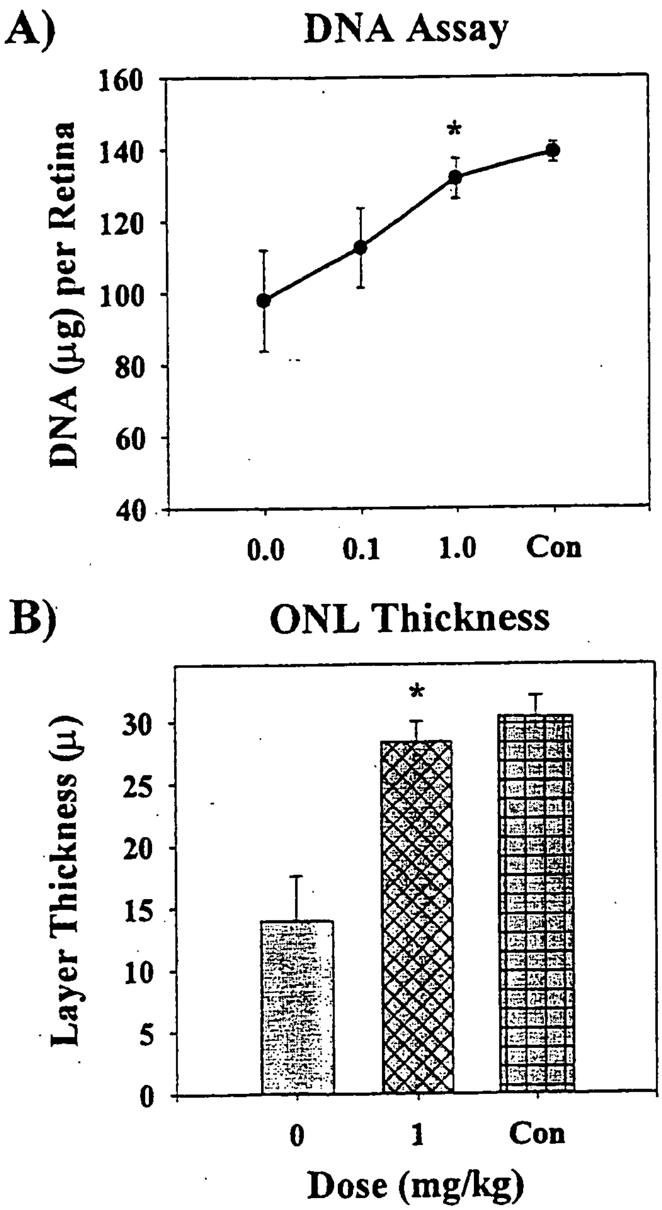
* Significantly higher responses compared to vehicles (p<0.05).

FIGURE 2



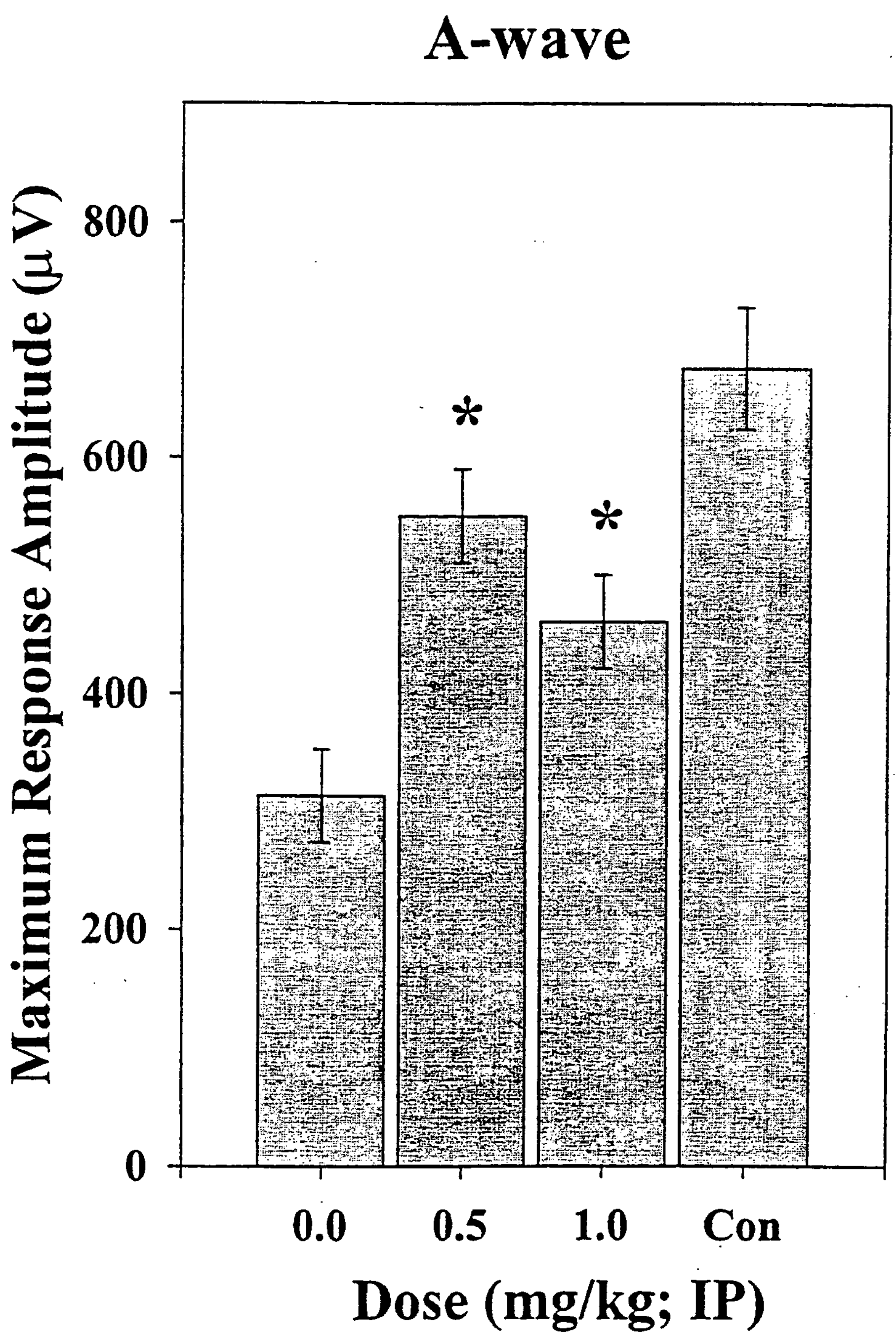
* Significantly less damaged than vehicle (p<0.05).

FIGURE 3



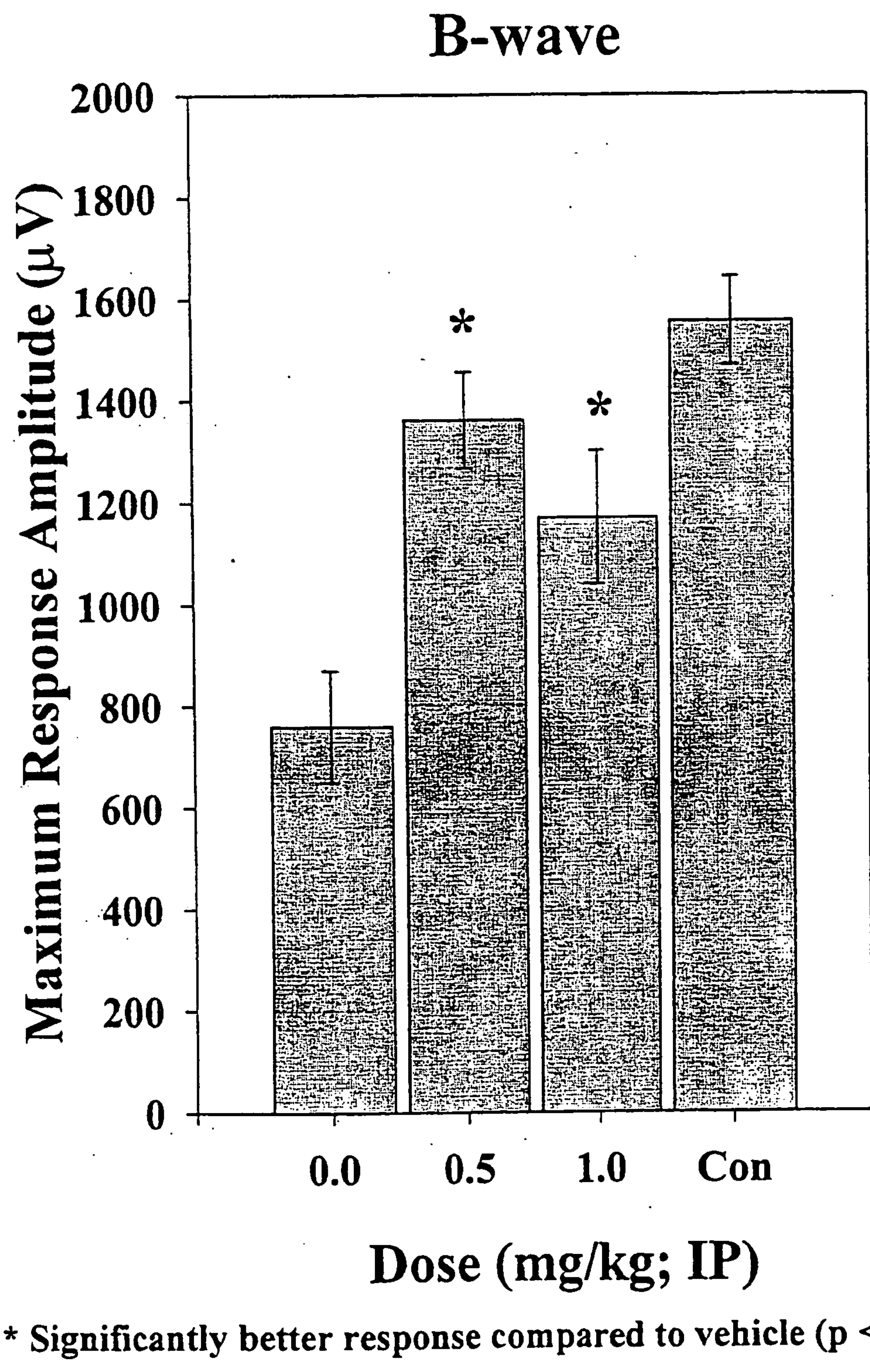
* Significantly better than vehicle (p<0.05) and not different than control.

FIGURE 4A



* Significantly better response compared to vehicle ($p < 0.05$).

FIGURE 4B



* Significantly better response compared to vehicle ($p < 0.05$).

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